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Original Article

Amelioration of female menopausal syndrome by intravenous administration of autologous menstrual blood-derived stem cells

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ABSTRACT

Introduction: Menopausal syndrome is characterized by a wide range of physical and psychological symptoms in women aged 40s–50s as a result of hormonal fluctuations and age-related decline. Various treatments have been used to manage the symptoms, including hormone replacement therapy, but no effective causal therapies have yet been identified. Regenerative medicine has gained considerable attention as a promising approach to age-related problems, and mesenchymal stem cell therapies have been extensively studied. Recently, menstrual blood has emerged as a novel cell source of stem cells, called menstrual blood-derived stem cells (MenSCs), due to its non-invasive, regular and consistent collection from women. In this study, we have investigated the therapeutic potential of intravenous administration of autologous MenSCs on female menopausal syndromes.
Methods: Menstrual blood was collected from 15 patients aged 30s–60s with ovarian dysfunction using a menstrual cup, and MenSCs were isolated, cultured and expanded. Patients received either 3×10^7 cells or 1×10^8 cells intravenously 1 to 5 times at intervals of more than 1 month. Patient-reported symptoms were assessed using the Simplified Menopausal Index at pre-treatment and after 1, 3, 6, and 12 months, and safety assessments were performed. Serum estradiol and follicle-stimulating hormone levels were also measured by immunoassay.
Results: Almost all patients who received MenSCs experienced a sharp reduction in menopausal symptoms, including vasomotor, neuropsychiatric, and motor symptoms, one month after the first administration, and these symptoms remained low for 6 months. The Simplified Menopausal Index score was significantly reduced after treatment. The reducing potency of 1×10^8 MenSCs was greater than that of 3×10^7 MenSCs. Patients who received a higher number of MenSCs showed an increasing trend in estradiol levels and a decreasing trend in follicle-stimulating hormone levels. When MenSCs were administered to postmenopausal patients, this trend was more pronounced. Overall, no apparent serious adverse events were observed during these treatments.
Conclusions: The present results suggest that the administration of MenSCs improved menopausal symptoms and regulated hormonal balance without any serious adverse events. This is the first report on

Abbreviations: E2, estradiol; FSH, follicle-stimulating hormone; HR, hormone replacement therapy; MenSC, menstrual blood-derived stem cell; MSC, mesenchymal stem cell; SMI, Simplified Menopausal Index.

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the promising therapeutic potential of cell-based therapy using autologous MenSCs for female menopausal syndrome.

1. Introduction

Menopausal syndrome is a complex clinical condition experienced by women in their 40s–50s as they approach the menopause, characterized by a wide range of physical and psychological symptoms resulting from hormonal fluctuations and age-related. Common symptoms include vasomotor disturbances such as hot flushed, sudden sweating and palpitations [1–3]. In addition, women often experience neuropsychological symptoms such as mood swings, difficulty concentrating, memory problems, anxiety, depression and headaches [4]. Sleep problems, including insomnia and poor sleep quality, are also common [2,3]. Moreover, urogenital symptoms such as vaginal dryness, dyspareunia, urinary frequency and urinary incontinence are commonly reported [5]. Musculoskeletal symptoms such as joint and muscle pain, fatigue and bone density loss leading to osteoporosis may occur [6]. Other notable effects include weight gain, reduced skin elasticity and hair thinning, which can have a significant impact on daily life [7].

A variety of treatments have been used to manage such a wide range of symptoms, including hormone replacement therapy (HRT), traditional herbal medicine (Kampo), placental injections, psychosomatic approaches, relaxation techniques and home-based exercise therapy. However, HRT has often been associated with an increased risk of women's cancers, such as breast, uterine and ovarian cancers, leading some patients to use HRT cautiously or avoid it altogether [8,9]. There is a growing need for novel therapeutic approaches that balance efficacy with patient safety. In recent years, new drugs such as elinzanetant and fezolinetant, which act as neurokinin-1 and neurokinin-3 receptor antagonists, have been developed. These agents suppress the hyperactivation of hypothalamic KNDy neurons, offering potential relief from vasomotor symptoms such as hot flushes and night sweats, as well as related problems such as nocturnal awakenings and sleep disturbances [10]. However, these treatments target specific symptoms and do not comprehensively address the wider range of menopausal symptoms, including the decline in ovarian function.

The ovaries are the earliest aging organs in the female reproductive system [11], highlighting the need for treatments that fundamentally restore hormonal balance. Regenerative medicine has gained considerable attention as a promising approach to addressing age-related problems. By promoting tissue repair and regeneration at the cellular level, regenerative medicine aims to improve the function of tissues compromised by aging or disease. Among various cell-based therapies, mesenchymal stem cell (MSC) treatments have been extensively studied in regenerative medicine [12–14]. In particular, menstrual blood has recently emerged as a novel cell source for stem cells called menstrual blood-derived stem cells (MenSCs), due to their non-invasive, regular and consistent collection from women, proliferative capacity, multipotency, anti-inflammatory properties and immunomodulatory functions [11]. Treatment with MenSCs has the potential to be a novel therapeutic option for menopausal symptoms, including ovarian dysfunction. Growth factors and cytokines secreted by these cells are thought to promote tissue regeneration and suppress inflammation, potentially helping to restore ovarian function and hormonal balance and alleviate complex menopausal symptoms. Furthermore, after administration, MSCs migrate to specific tissues or target sites, exert localized effects, undergo apoptosis, and are naturally metabolized and cleared [15]. This characteristic positions them as a potentially safe treatment option [16].

In this study, we have investigated the therapeutic potential of intravenous administration of autologous MenSCs into patients with female menopausal syndrome. The administration of MenSCs improved menopausal symptoms and regulated the hormonal balance without any serious adverse events. To the best of our knowledge, this is the first report on the promising therapeutic effects of the cell-based therapy using autologous MenSCs against female menopausal syndrome.

2. Methods

2.1. Ethical approval

This study protocol has been approved by the Specific Certified Regenerative Medicine Committee of the Japanese Society of Skin Regenerative Medicine (certification number: PB3200082) and submitted to the Ministry of Health, Labour and Welfare. Written informed consent was obtained from each patient, and the treatment was performed in accordance with the Act on Securing Safety of Regenerative Medicine.

2.2. Study period and patient selection

Autologous MenSC therapy was conducted from the date of the treatment plan on August 24, 2020 until October 31, 2024. This study included 15 patients (30 treatments in total) aged 30s–60s with ovarian dysfunction in the female menopausal syndrome (Table 1). Inclusion and exclusion criteria are shown in Table 2.

2.3. Menstrual blood collection

A medical-grade menstrual cup made of silicone rubber, which is flexible and funnel-shaped, was inserted into the vagina during menstruation (Fig. 1A–C). Approximately 10~20 mL of menstrual blood was collected transferred to a container pre-filled with antibiotics (penicillin, streptomycin, and amphotericin B, Wako) and anticoagulant (heparin, Mochida Pharmaceutical), stored in a cold pack, and delivered to the clinic within 72 h (Fig. 1D and E).

2.4. Preparation of MenSCs

MenSCs were prepared from menstrual blood. Briefly, menstrual blood samples were washed with phosphate buffered saline containing antibiotics and anticoagulants. Mononuclear cells were isolated from the menstrual blood samples by a density gradient centrifugation using Lymphoprep (Serumwerk Bernburg AG), and further purified by reaction with magnetic microbead-conjugated antibodies against CD34, CD45, and CD105 (Miltenyi Biotec), followed by passing through an LS column (Miltenyi Biotec) using MidiMACS Separator (Miltenyi Biotec). The resulting $CD34^{-}CD45^{-}CD105^{+}$ cells were cultured in Dulbecco's modified Eagle's medium:Ham's F-12 nutrient mixture (1:1, Wako)

Table 1
Patient information and clinical data. Patients a~d were received 1×10^8 MenSCs, Patients e ~ o were received 3×10^7 MenSCs.

Patient	Age (years)	Number of treatments	Dosing interval	Menopausal status	Collection method	HRT treatment
a	42	3	1 year 2 years	Pre	Menstrual blood	No
b	41	1		Pre	Menstrual blood	No
c	46	1		Pre	Menstrual blood	No
d	39	2	7 months	Pre	Menstrual blood	No
e	44	3	1 month 3 months	Pre	Menstrual blood	Yes
f	48	2	4 months	Pre	Menstrual blood	Yes
g	44	1		Pre	Menstrual blood	No
h	46	5	1 month 4 months 2 months 2 months	Pre	Menstrual blood	No
i	68	3	Monthly	Post	Hormone-induced bleeding	No HRT: None post-intravenous
j	41	2	1 month	Pre	Menstrual blood	No
k	49	3	Monthly	Pre	Hormone-induced bleeding	Yes
l	31	2	2 months	Pre	Menstrual blood	No
m	35	2	1 month	Pre	Menstrual blood	No
n	54	1		Post	Hormone-induced bleeding	No HRT: None post-intravenous
o	37	1		Pre	Menstrual blood	No

supplemented with 5 % autologous serum isolated from own peripheral blood and antibiotics, expanded, and stored as MenSCs at −80 °C or in liquid nitrogen until use. Quality testing was performed prior to treatment, including visual inspection, sterility testing, cell count and viability testing, mycoplasma testing and endotoxin testing to ensure safety. Phase contrast microscopy images of cell morphology and growth are shown in Fig. 2A. Cells were stained using the BD Stemflow Human MSC Analysis Kit [APC mouse anti-human CD73 (clone AD2), FITC mouse anti-human CD90 (clone 5E10) and PerCP-Cy5.5 mouse anti-human CD105 (clone 266), BD Biosciences] and analyzed using a Coulter EPICS XL-MCL (Beckman) and the Kaluza software (Beckman). The purity of cells positive for CD73, CD90, and CD105 was more than 98 % (Fig. 2B).

Table 2
Key inclusion and exclusion criteria for receiving MenSC treatment.

Selection Criteria	
1	Patients for whom intravenous administration of autologous MenSCs is considered an effective means of treating their main complaint or fulfilling their hopes and expectations.
2	Patients with diseases or syndromes primarily characterized by ovarian dysfunction, such as menopausal disorders (menopausal syndrome), menstrual irregularities, infertility or ovarian insufficiency, for whom other treatments are not considered to be sufficiently effective or who may be undergoing treatment for these conditions.
3	Patients in good general health.
4	In the case of minors, those who have the consent of a legal representative.
5	Patients who have given written consent (or consent from a legal representative in the case of minors).
Exclusion Criteria	
1	Absence of Menstruation at the Time of Cell Collection: Cases where menstruation stops for reasons such as menopause or amenorrhea. Note: Withdrawal bleeding or hormone-induced bleeding is acceptable.
2	First Menstrual-like Bleeding Postpartum or After Miscarriage: The first menstrual-like bleeding after childbirth or miscarriage. Note: Approximately one month after childbirth or miscarriage, patients may report bleeding that is clinically difficult to distinguish from normal menstruation. This ensures that contamination by residual fetal-derived tissue is excluded.
3	Sexual Activity Prior to Menstrual Blood Collection: Sexual intercourse occurring between the last menstrual period and the current menstrual blood collection. Note: Ensures no contamination by sperm from a third party.
4	Inability to Attend Follow-up Visits: Patients who cannot regularly visit the clinic during or after the treatment.
5	Lack of Informed Consent: Patients who do not provide written consent for the treatment.
6	Positive Test Results for Certain Infections: Includes syphilis, HBV, HCV, HIV, or genital infections. Note: Patients with HBV infection may be treated if consent is obtained.
7	Pregnancy: Pregnant patients are excluded.
8	History of Allergic Reactions to Treatment: Patients who have previously experienced allergic reactions related to this treatment.
9	High Risk of Blood Disorders or Infections: Patients identified on pre-treatment assessment as having or suspected of having sepsis, bleeding tendency, or high-risk hematological disorders.
10	Other Conditions Deemed Unsuitable by the Physician: Any other cases where the treating physician does not consider the treatment to be appropriate.

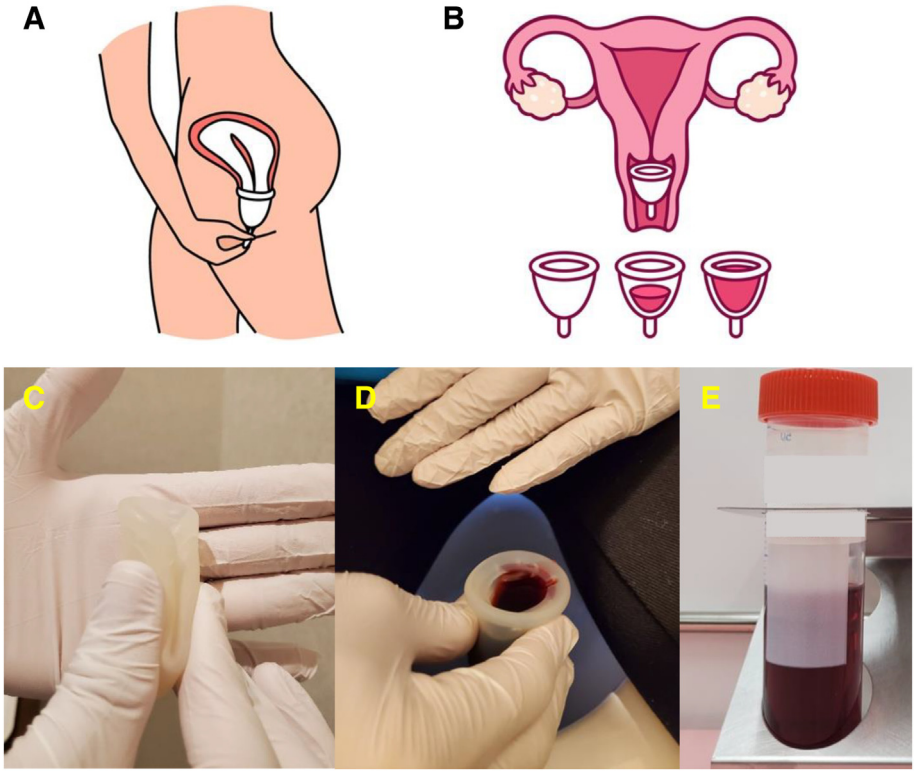

Fig. 1. Non-invasive menstrual blood collection technique using a menstrual cup. (A, B) A medical grade menstrual cup made of silicone rubber and flexible funnel-shaped (C) was inserted into the vagina during menstruation. Menstrual blood (10–20 mL) was collected for the preparation of MenSCs (D, E).

2.5. Intravenous administration of MenSCs

Patients were intravenously administered with either different cell numbers of MenSCs, 3×10^7 cells or 1×10^8 cells, in 100 mL saline containing 1 % autologous serum over 30–60 min, taking into account the general condition of the patient. The number of administrations was adjusted according to the patient's condition, with an interval of at least 1 month between treatments to ensure

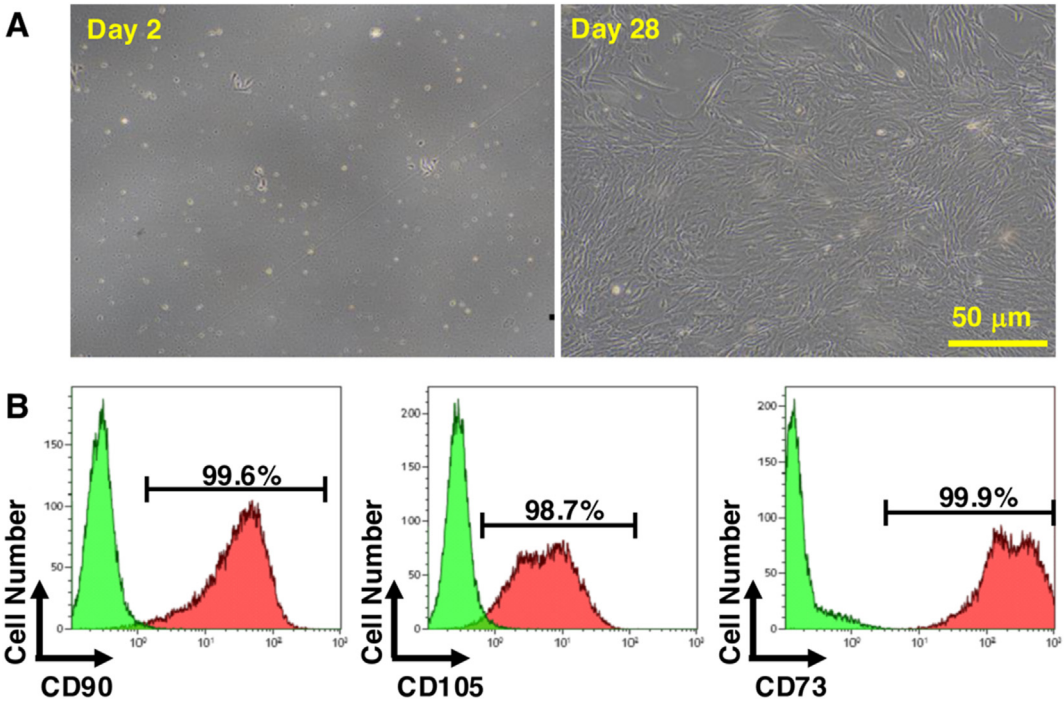

Fig. 2. Cell morphology and purity of MenSCs. Phase-contrast microscopy images of MenSCs on day 2 and day 28 after the cell culture initiation are shown (A). Flow cytometry analysis of MenSCs was performed to determine the purity of cells positive for CD73, CD90, and CD105 and representative data are shown (B).

safety. For 1×10^8 MenSCs, dosing intervals ranged from 7 months to 2 years, and for 3×10^7 MenSCs, dosing intervals ranged from 1 to 4 months (Table 1). MenSCs (1×10^8 cells) were administered to four patients a~d in a total of 6 treatments (1 treatment for two patients, 2 treatments for one patient and 3 treatments for one patient). MenSCs (3×10^7 cells) were administered to eleven patients e ~ o in a total of 24 treatments (1 treatment for three patients, 2 treatments for four patients, 3 treatments for three patients and 5 treatments for one patient). Of the eleven patients, two patients experienced withdrawal bleeding due to hormone replacement therapy (HRT), which was stopped after the start of the cell collection treatment.

2.6. Efficacy and safety evaluation

To assess treatment efficacy, patient-reported symptoms were assessed using the Simplified Menopausal Index (SMI, Table 3) [17] at pre-treatment and 1, 3, 6, and 12 months post-treatment. Follow-up assessments were also carried out to monitor illnesses, infections, allergic reactions, embolisms and fevers for safety assessments. The scientific validity of the treatment was comprehensively evaluated by measuring the patients' serum estradiol (E2) and follicle-stimulating hormone (FSH) levels using electrochemiluminescence immunoassay and chemiluminescent immunoassay, respectively, by SRL, Inc. (Japan).

2.7. Statistical analysis

Data are described as the mean ± standard deviation (SD) for each group. Statistical analysis was performed with the GraphPad Prism software (version 7.05; GraphPad Software) using one-way analysis of variance with Dunnett's multiple comparison test for comparisons involving three groups. $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Intravenous administration of MenSCs improves menopausal symptoms

During the treatment, individual symptoms, including vasomotor, neuropsychiatric, and motor symptoms, were assessed over time in each patient (Fig. 3). Patients a~d who received 1×10^8 MenSCs showed an immediate decreasing trend in vasomotor symptom scores, including hot flushes and sweating, 1 month after the first administration compared to pre-treatment levels (Fig. 3A). These symptoms remained low for 6 months, but one patient showed an increase at 12 months. Patients e ~ o who received 3×10^7 MenSCs also showed a decreasing trend in score over 6 months after administration, except for two patients who showed a transient increase in score at 1 month, followed by a decrease over the next 6 months (Fig. 3B). However, two patients showed an increase in score at 12 months. In terms of neuropsychiatric symptom scores, including anxiety and depression which are more common in women, patients a~d who received 1×10^8 MenSCs showed a significant decrease in scores over 6 months after the first administration compared to pre-treatment levels (Fig. 3C). However, one patient showed an increase in score at 12 months. Patients e ~ o who received 3×10^7 MenSCs also showed a significant decrease in score over 12 months (Fig. 3D). In addition, in terms of motor symptom scores, including muscle weakness and joint pain, patients a~d who received 1×10^8 MenSCs showed an immediate trend towards a reduction in scores up to 12 months (Fig. 3E). Patients e ~ o who received 3×10^7 MenSCs showed a similar decreasing trend in score for up to 12 months, with the exception of one patient who showed an increase at 1 month, followed by a decrease over 6 months (Fig. 3F).

Overall, the menopausal symptom score of a total of 15 patients who received 1×10^8 or 3×10^7 MenSCs tended to show an immediate reduction in vasomotor, neuropsychiatric and motor symptoms 1 month after the first administration. These symptoms then remained low for 6 months. The SMI score, which aggregates these individual symptom scores, was significantly reduced after treatment with both 1×10^8 and 3×10^7 MenSCs (Fig. 3G and H), but the reduction was more pronounced when the higher number of cells was administered. Thus, MenSCs therapy has a comprehensive effect in alleviating female-specific symptoms.

3.2. Intravenous administration of MenSCs improves female hormone balance

Next, hormone levels were measured before and after treatment (Fig. 4). In four patients who received 1×10^8 MenSCs, an increasing trend in E2 levels was observed as early as 1 month after treatment (Fig. 4A). Similarly, all but one of the nine patients who received 3×10^7 MenSCs showed an upward trend in E2 levels at the final follow-up point (Fig. 4B). In contrast, a decreasing trend in FSH levels was observed in two patients who received 1×10^8 MenSCs (Fig. 4C) and six patients who received 3×10^7 MenSCs (Fig. 4D) at the final follow-up point.

Since we cannot exclude the possibility that these values are due to random variations caused by menstrual cycle fluctuations in premenopausal women, we next focused on the data from two postmenopausal patients i and n, even without the use of HRT. Administration of MenSCs to these patients more clearly showed an increasing trend in E2 levels (Fig. 5A) and a decreasing trend in FSH levels (Fig. 5B).

Table 3
SMI score for evaluation of the menopausal symptoms. Questions 1–4 are related to vasomotor symptoms, questions 5–8 are related to neuropsychiatric symptoms, and questions 9,10 are related motor symptoms. The score for each symptom was calculated by summing the scores for the individual questions. The SMI was calculated by summing the three symptom scores.

Question	Symptom	Symptom Severity (Score)			
		Severe	Moderate	Mild	None
1	Hot flush	10	6	3	0
2	Increased Sweating	10	6	3	0
3	Cold sensitivity in the back and extremities	14	9	5	0
4	Shortness of breath and palpitations	12	8	4	0
5	Shortness of breath and palpitations	14	9	5	0
6	Increased irritability and a tendency to anger	12	8	4	0
7	Frequent worrying and depressive moods	7	5	3	0
8	Frequent headaches, dizziness, and nausea	7	5	3	0
9	Increased fatigue	7	4	2	0
10	Shoulder tension, back pain, and limb pain	7	5	3	0

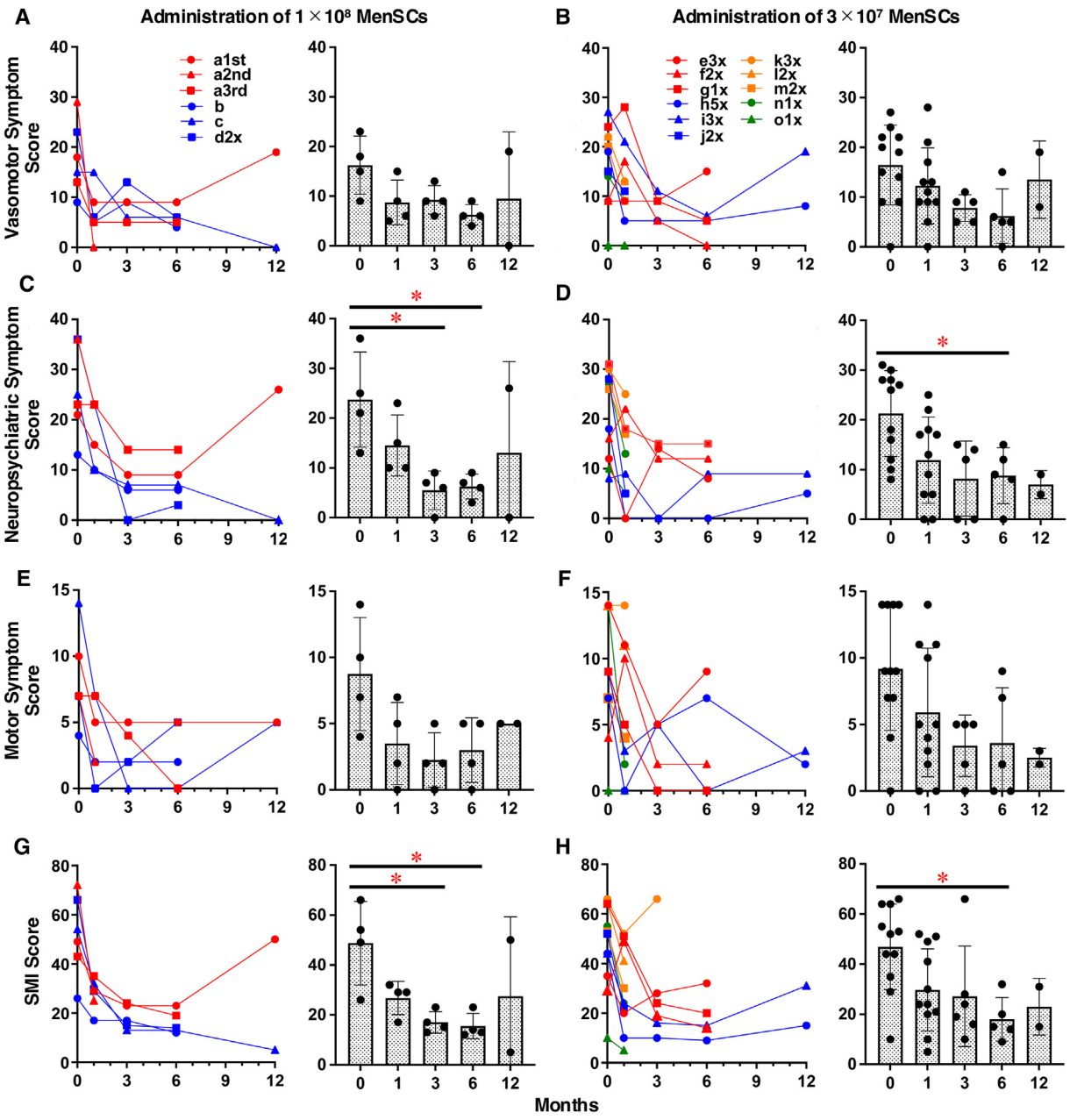

Fig. 3. Intravenous administration of MenSCs improves menopausal symptoms. Menopausal symptoms including vasomotor symptoms (A, B), neuropsychiatric symptoms (C, D), motor symptoms (E, F) and SMI (G, H) were assessed over time following administration of 1×10^8 (A, C, E, G) or 3×10^7 (B, D, F, H) autologous MenSCs. Changes in each score over time for individual patients are shown on the left and the statistical results are shown on the right. The time point at 0 month indicates the pre-treatment baseline value. Patient a1st, a2nd and a3rd represent the same patient a who received 1×10^8 MenSCs three times at intervals of 1 and 2 years, respectively. Only data from a1st among them were used for statistical analysis. For patients e ~ o, 1 × ~5 × means that each patient received 3×10^7 MenSCs 1 to 5 times at intervals of 1~4 months (Table 1). Data are shown as the mean ± SD [$n = 4$ (A, C, E, G), $n = 11$ (B, D, F, H)], and *P*-values were determined by one-way analysis of variance with the Dunnett's multiple comparisons test. $*P < 0.05$, $**P < 0.01$.

These results suggest that improving hormonal balance in postmenopausal patients can be achieved without relying on external hormone supplementation. This makes it an important option for patients who want to minimize the risk of hormone-related side effects.

3.3. No serious adverse events are observed with treatment of MenSCs

Safety outcomes such as thrombosis, infections, allergic reactions and fever were monitored during treatment. No serious adverse events were observed in any of the cases. In particular, safety was confirmed in the group receiving repeated doses of 3×10^7 cells, supporting the clinical significance of treatment safety and sustainability.

4. Discussion

This report highlights the potential efficacy of intravenous administration of autologous MenSCs in relieving menopausal symptoms and improving ovarian function. These results show that intravenous administration of MenSCs effectively improves menopausal symptoms and ovarian function decline. Intravenous administration of MenSCs tended to increase E2 levels and decrease

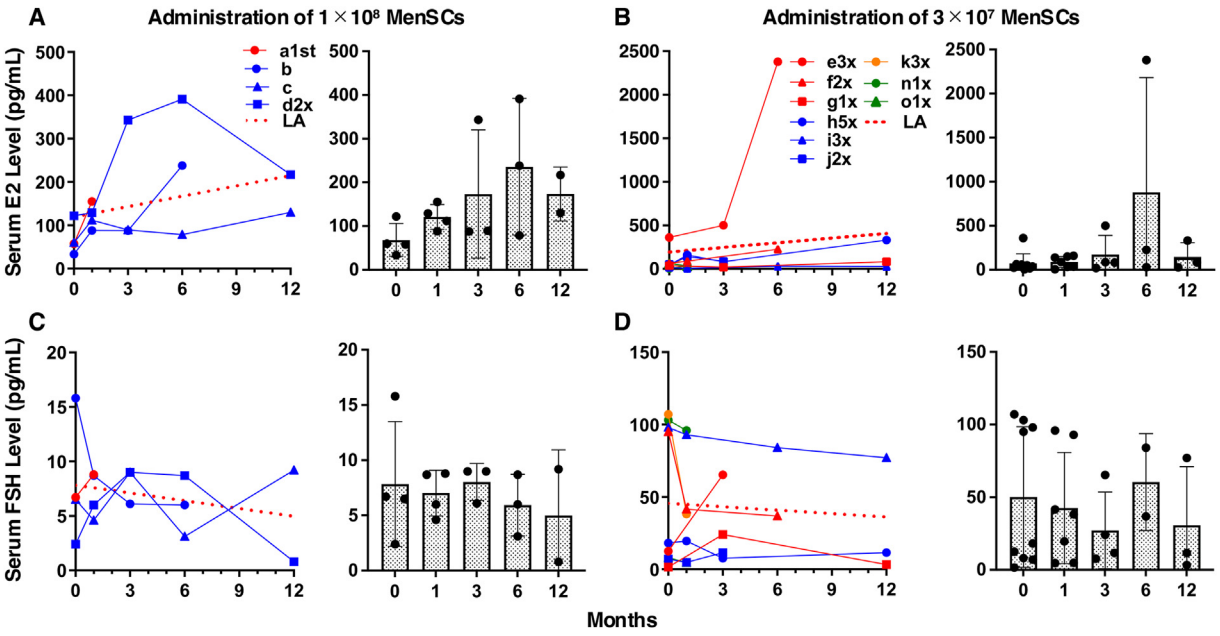

Fig. 4. Intravenous administration of MenSCs improves female hormonal balance. Serum E2 levels (A, B) and FSH levels (C, D) were determined over time after administration of 1×10^8 (A, C) or 3×10^7 (B, D) autologous MenSCs. Changes in each serum level over time for individual patients are shown on the left and the statistical results are shown on the right. The time point at 0 month indicates the pre-treatment baseline values before treatment. Data are shown as the mean ± SD [$n = 4$ (A, C, E, G), $n = 9$ (B, D, F, H)], and *P*-values were determined by one-way analysis of variance with the Dunnett's multiple comparisons test. LA, linear approximation.

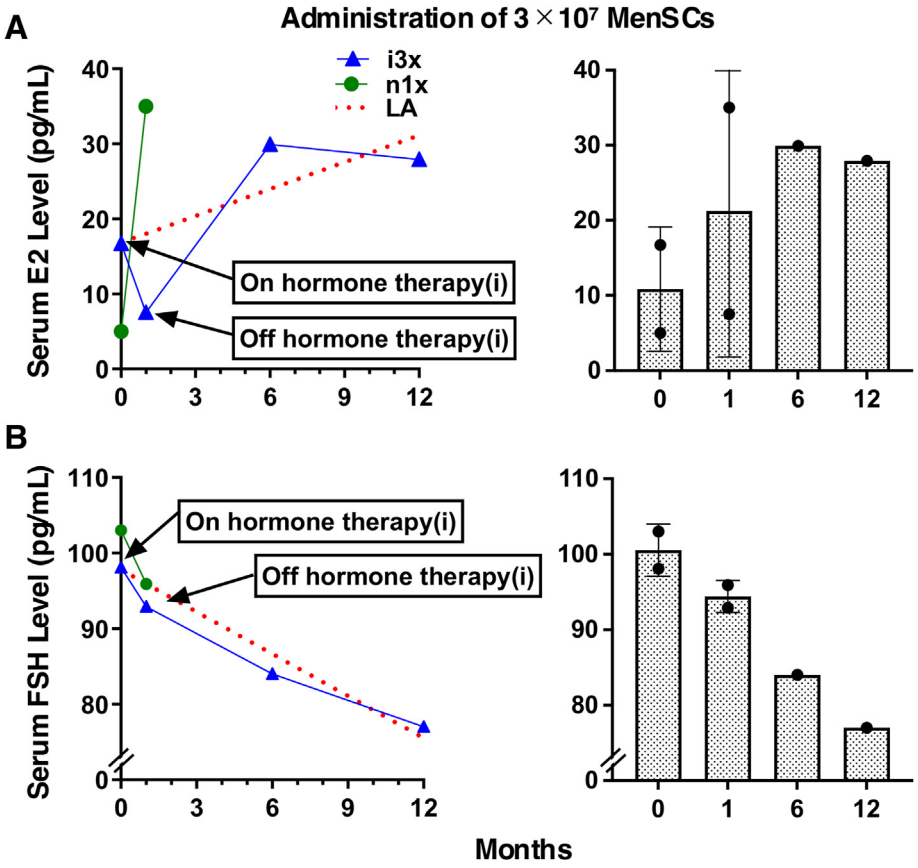

Fig. 5. Intravenous administration of MenSCs improves female hormonal balance in postmenopausal patients (i and n). Serum E2 levels (A) and FSH levels (B) were determined over time after administration of 3×10^7 autologous MenSCs. Changes in each serum level over time for individual patients are shown on the left and the statistical results are shown on the right. The time point at 0 month indicates the pre-treatment baseline values before treatment. Data are shown as the mean ± SD ($n = 2$). LA, linear approximation.

FSH, suggesting the potential for partial restoration of endogenous hormone regulation and ovarian function. While an increase in ovarian mass following intravenous administration has been reported in mouse models of ovarian failure [18], to our knowledge, this is the first clinical report demonstrating such effects in humans. These findings suggest a novel therapeutic approach that differs from traditional HRT in that it promotes the patient's own hormone secretion. Taken together, these results represent a significant advance in regenerative medicine research by expanding the use of non-invasive and sustainable stem cell sources for therapeutic purposes.

In this study, the administration strategy was defined with two dose levels: a high dose of 1×10^8 cells and a low dose of 3×10^7 cells. This classification is based on the assumption that the effects of intravenous cell transplantation may be temporary. Therefore, multiple administrations may help to achieve sustained therapeutic benefit. Regarding the optimal cell dose and frequency of administration, the previous paper on 16 clinical trials of intravenous MSC administration suggested an effective dose range of 1×10^8 to 1.5×10^8 cells per infusion [16]. Based on this, we administered 1×10^8 cells per treatment or 3×10^7 cells per treatment 3 times. One patient h who received five relatively evenly spaced doses of 3×10^7 MenSCs maintained marked improvements in vasomotor, psychological and motor symptoms for up to 12 months after starting treatment (Fig. 3B–D, F, H). This suggests that multiple lower doses of 3×10^7 MenSCs given at regular intervals could reduce SMI scores and improve symptoms. However, the number of treatments is influenced by the patients' schedule and lifestyle. Compared to the single administration of 1×10^8 MenSCs, the multiple administration of 3×10^7 MenSCs may make it difficult to establish a consistent treatment schedule due to variable patient availability (Fig. 3). Therefore, although multiple administrations of 3×10^7 MenSCs would help reduce risks such as embolism while maximizing therapeutic effects and duration of symptom relief, overall, a single administration of higher dose of 1×10^8 MenSCs would be effective and benefit patients if sufficient numbers of MenSCs can be prepared. Further research into more flexible and personalized treatment protocols is needed.

Intravenous administration exerts systemic therapeutic effects rather than being confined to specific organs, making it particularly effective for conditions such as menopausal symptoms, which involve multiple problems at the same time. Menopausal symptoms manifest in several areas, including the vasomotor (e.g., sweating, hot flushes), psychological (e.g., anxiety, depression), and motor systems (e.g., joint pain, muscle weakness) [17], requiring a comprehensive systemic approach to treatment. In addition to intravenous delivery, localized delivery options may increase the flexibility of MenSC-based therapies. These methods directly target specific organs, potentially improving therapeutic outcomes [19,20]. By appropriately combining local and intravenous administration, it is hoped that a treatment strategy can be developed to improve both systemic and localized symptoms simultaneously. Thus, the application of MenSC therapy is not limited to menopausal symptoms but also has the potential to be extended to various conditions such as infertility treatment, gynecological disorders, multi-organ failure and chronic inflammatory diseases. Combining flexibility and versatility, this therapy has the potential to become a new foundation for personalized medicine, offering hope to many patients.

The benefits of MenSC therapy go beyond relieving menopausal symptoms and may help maintain bone density and reduce the risk of cardiovascular disease. In particular, improved estrogen secretion may promote bone formation and help prevent osteoporosis and related conditions [21]. Improving mental health is another important effect of estrogen. Estrogen regulates neurotransmitters such as serotonin and dopamine, and its decline is associated with increased anxiety and depression [22]. The present study revealed the elevated E2 levels following MenSC administration, suggesting potential improvements in neurotransmitter metabolism and mental stability. The psychological benefits observed with intravenous administration of MenSC may alleviate the psychological distress associated with menopausal symptoms and significantly improve patients' quality of life. This effect may be particularly beneficial in postmenopausal women. Estrogen improves the contractility of vascular smooth muscle cells, enhances elastin fiber formation and increases the expression of cell adhesion proteins [23]. Exosomes released from MenSCs have also been shown to promote angiogenesis and activate critical signaling pathways [24]. These effects suggest the potential to reduce the risk of cardiovascular diseases such as atherosclerosis and hypertension, while improving vascular health and longevity.

MSCs are adult stem cells with the capacity for self-renewal and multipotent differentiation, and their many clinical trials have been conducted worldwide [12–14,25,26]. The first report on MenSCs was published in 2007 by Meng et al. [27]. MenSCs are also known by various other names, including menstrual-derived stem cells, menstrual blood stem cells, endometrial stem cells, menstrual blood-derived endometrial stem cells and menstrual blood-derived mesenchymal stem cells [28]. MenSCs provide an alternative source of adult stem cells for research and applications in regenerative medicine [28]. Unlike bone marrow- and adipose-derived stem cells, MenSCs are naturally shed from the body, making them non-invasive from a collection standpoint. This property has led to high expectations for their use in various regenerative medicine therapies [29]. In addition, MenSCs can differentiate into a variety of cell types, including cardiac, neural, bone, cartilage and adipose cells. No cases of teratoma formation, ectopic tissue development or immune reactions have been reported after transplantation in animal models. Thus, their cellular plasticity and safety have been demonstrated in several studies [28]. In addition to the direct effects of cell transplantation, MenSCs exert extensive therapeutic effects beyond mere cell transplantation through the secretion of growth factors, cytokines, and extracellular vesicles (e.g., exosomes) [30]. These secreted substances include several bioactive molecules, such as vascular endothelial growth factor, which promotes angiogenesis, and interleukin-10, which suppresses inflammation [31,32]. Exosomes are small extracellular vesicles, ranging from 30 to 150 nm in diameter, secreted by various cell types, including MSCs [31]. These vesicles play an important role in intercellular communication by transferring bioactive molecules such as proteins, lipids, mRNAs and microRNAs to recipient cells, therby influencing their function. Exosomes can suppress immune responses, reducing the risk of post-transplant rejection and being a key factor in realizing systemic therapeutic effects [33]. Therefore, through the action of these bioactive molecules secreted by MenSCs [34], MenSC therapy has the potential to restore hormonal balance and provide a systemic anti-aging treatment option. The underlying mechanism may involve the possible expression of embryonic stem cell-like markers such as Oct4, SSEA and Nanog in MenSCs, but further research is needed to elucidate this possibility [35].

The importance of using MenSCs as a stem cell source goes beyond conventional cell therapy. The non-invasive collection of menstrual blood, a biological material that is often discarded, using menstrual cups [36,37], opens new avenues for improving the sustainability of medical resources. This approach is very safe because it uses cells collected from the patient and is also excellent from an ethical point of view [38]. The non-invasive collection of MenSCs has attracted attention for its potential to overcome the challenges associated with traditional stem cell therapies. Despite advances in collection techniques, adipose-derived stem cells and bone marrow-

derived stem cells still require invasive surgical procedures. These methods are physically demanding for patients and carry risks such as infection and pain at the collection site [39,40]. In contrast, menstrual blood is regularly expelled by women of reproductive age, and the collection process involves minimal physical and psychological strain. In addition, because menstrual blood can be collected regularly and consistently, it offers high reproducibility and sustainability of treatment, further enhancing its potential in regenerative medicine [29]. This feature allows multiple cell collections from the same patient, enabling the development of long-term treatment plans. Moreover, harvested cells can be cryopreserved [41] and thawed when needed. Advances in cryopreservation technology greatly increase the flexibility of cell-based therapies, making it a critical factor in their practical application. Previous studies have shown that menstrual blood-derived stem cells expanded under standard culture conditions typically do not express HLA-DR, consistent with the broader MSC profile [27,42]. In this study, we did not assess HLA-DR expression, it is important to confirm the immunophenotype in future investigations. The use of MenSCs is thus significant for its medical benefits and its potential to improve the efficient use of medical resources and enhance ethical standards in healthcare. This feature is critical to extending the applicability of regenerative medicine to broader patient population, thereby promoting its wider adoption.

For the practical implementation of MenSC therapy, more research is needed. In particular, it is essential to elucidate the specific mechanisms of action of the cytokines, growth factors and extracellular vesicles secreted by these cells, to establish optimal dosing protocols and to confirm efficacy and safety in large-scale clinical trials. Additionally, reducing the cost of treatment and developing standardized cell culture technologies are essential to make this therapy available to more patients. The results of this study suggest that MenSC therapy could usher in a new era in women's health. This therapy has the potential to the quality of life of postmenopausal women and open up new horizons in the future of regenerative medicine. Further research and clinical application of MenSC therapy is expected to benefit more patients and expand the role of regenerative medicine in healthcare.

5. Conclusions

This is the first report to demonstrate the promising potential of autologous MenSC therapy to improve menopausal symptoms and ovarian function. Intravenous administration of MenSCs has been shown to reduce the severity of menopausal symptoms and improve hormonal balance (increase in E2, decrease in FSH). In addition, no serious adverse events were observed, indicating that this is a very safe treatment. These results suggest that this treatment could be a non-invasive, personalized and sustainable alternative to conventional treatments such as hormone replacement therapy. In the future, larger-scale clinical trials will be needed to determine the optimal dosage and administration protocol for long-term efficacy and safety. This research lays the groundwork for expanding the clinical application of MenSCs in regenerative medicine and women's health.

Declaration of competing interest

The authors have no conflicts of interest to declare. Hiromi Izawa is the director of Jingu-Gaien Woman Life Clinic, where the study was conducted.

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